

WHAT IS CLAIMED IS:

*SUM C1*

1. A method of increasing the mutation rate of a virus, comprising administering an RNA nucleoside analog to a virally infected cell, wherein the analog is incorporated by a polymerase into an RNA copy of a genomic nucleic acid encoding the virus, said analog replacing a first natural occurring nucleotide having a first complementary nucleotide wherein said analog complements a second nucleotide which is other than the first nucleotide, thereby inducing the virus to mutate.

10 2. The method of claim 1, wherein the RNA nucleoside analog replaces uracil.

15 3. The method of claim 1, wherein the RNA nucleoside analog replaces adenine.

4. The method of claim 1, wherein the RNA nucleoside analog replaces cytidine.

20 5. The method of claim 1, wherein the RNA nucleoside analog replaces guanine.

6. The method of claim 1, wherein the RNA nucleoside analog is incorporated by the polymerase into the RNA copy of the genomic nucleic acid with an efficiency at least about 0.1% that of a naturally occurring complementary nucleic acid.

25 7. The method of claim 1, wherein the method further includes the proviso that the RNA nucleoside analog is not ribavirin or a 5-halo analog of L- $\alpha$ -D-ribofuranosylimidazole-4-carboxamide.

30 8. The method of claim 1, wherein the RNA analog is a non-chain terminating analog.

9. The method of claim 1, wherein the method further includes the proviso that if the virus is HIV, then the RNA nucleoside analog is not HEPT or a 2',5'-bis-O-sialylated-3'-spiro-substituted (TSAO) adenine, hypoxanthine, N<sup>1</sup>-alkyl-hypoxanthine, or xanthine or a nucleoside analog that is incorporated and extended at high efficiency by reverse transcriptase of HIV.

10. The method of claim 1, wherein the nucleoside analog is selected from the group consisting of N<sup>4</sup>-aminocytidine, N<sup>1</sup>-methyl-N<sup>4</sup>-aminocytidine, 3,N<sup>4</sup>-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N<sup>4</sup>-dimethylcytidine, 5-(2-hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N<sup>4</sup>-methyl-N<sup>4</sup>-aminocytidine, 5-aminocytidine, 5-nitrosocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O<sup>2</sup>-methyluridine, O<sup>2</sup>-ethyluridine, 5-aminouridine, O<sup>4</sup>-methyluridine, O<sup>4</sup>-ethyluridine, O<sup>4</sup>-isobutyluridine, O<sup>4</sup>-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-(thioalkyl)-uridine, 1,N<sup>6</sup>-ethenoadenosine, 3-methyladenosine, and N<sup>6</sup>-methyladenosine, 8-hydroxyguanosine, O<sup>6</sup>-methylguanosine, O<sup>6</sup>-ethylguanosine, O<sup>6</sup>-isopropylguanosine, 3,N<sup>2</sup>-ethenoguanosine, O<sup>6</sup>-alkylguanosine, 8-oxoguanosine, 2,N<sup>3</sup>-ethenoguanosine, and 8-aminoguanosine.

11. The method of claim 1, wherein the virus is a retrovirus or a flavivirus.

20

12. The method of claim 11, wherein the virus is a pestivirus.

25

13. The method of claim 1, wherein the polymerase is a human polymerase II.

14. The method of claim 1, wherein the cell is in cell culture.

15. The method of claim 1, wherein the cell is in an animal.

30

16. The method of claim 1, wherein increasing the mutation rate of the virus produces a progressive loss of viability of the virus.

17. The method of claim 1, comprising administration of more than one species of RNA nucleoside analog to the virally infected cell.

5        18. The method of claim 1, wherein the virus is an RNA virus selected from the group consisting of hepatitis C, coronavirus, influenza, respiratory syncytial virus, BVDV, and dengue fever.

10      19. A viral particle comprising viral genomic RNA, wherein the viral genomic RNA comprises an RNA nucleoside analog.

20.     The viral particle of claim 19, wherein the particle is an HIV particle, and HCV particle, or a BVDV particle.

15      21. The viral particle of claim 19, wherein the nucleoside analog is selected from the group consisting of N<sup>4</sup>-aminocytidine, N<sup>1</sup>-methyl-N<sup>4</sup>-aminocytidine, 3,N<sup>4</sup>-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N<sup>4</sup>-dimethylcytidine, 5-(2-hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N<sup>4</sup>-methyl-N<sup>4</sup>-aminocytidine, 5-aminocytidine, 5-nitrosocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O<sup>2</sup>-methyluridine, O<sup>2</sup>-ethyluridine, 5-aminouridine, O<sup>4</sup>-methyluridine, O<sup>4</sup>-ethyluridine, O<sup>4</sup>-isobutyluridine, O<sup>4</sup>-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-(thioalkyl)-uridine, 1,N<sup>6</sup>-ethenoadenosine, 3-methyladenosine, and N<sup>6</sup>-methyladenosine, 8-hydroxyguanosine, O<sup>6</sup>-methylguanosine, O<sup>6</sup>-ethylguanosine, O<sup>6</sup>-isopropylguanosine, 3,N<sup>2</sup>-ethenoguanosine, O<sup>6</sup>-alkylguanosine, 8-oxo-guanosine, 2,N<sup>3</sup>-ethenoguanosine, and 8-aminoguanosine.

22. A population of cells comprising a highly variable population of replicated homologous viral nucleic acids.

30      23. The population of cells of claim 22, wherein the cells are in cell culture.

24. The population of cells of claim 22, wherein the viral nucleic acids are derived from a retrovirus or a flavivirus.

5 25. A cell comprising a viral genomic nucleic acid, an RNA analog, a cellular mRNA analog and a viral genomic RNA analog.

10 26. The cell of claim 25, wherein the viral genomic nucleic acid is integrated into the cellular genome.

15 27. The cell of claim 25, wherein the viral genomic nucleic acid is a retroviral or a flaviviral nucleic acid.

20 28. The cell of claim 25, wherein the viral genomic nucleic acid is an HIV nucleic acid, a pestivirus nucleic acid, or an HCV virus nucleic acid.

25 29. The cell of claim 25, wherein the viral genomic nucleic acid is selected from the group consisting of a HIV-1, HIV-2, HTLV-1, HTLV-2, SIV, hepatitis A, hepatitis B, hepatitis C, BVDV, and dengue fever virus.

30 30. A method of detecting the mutagenic potential of a ribonucleoside analog comprising integrating the ribonucleoside analog into a viral RNA synthesized by a polymerase, and determining whether the incorporation causes a mutation in a progeny virus.

35 31. The method of claim 30, wherein the ribonucleoside analog is selected from the group consisting of N<sup>4</sup>-aminocytidine, N<sup>1</sup>-methyl-N<sup>4</sup>-aminocytidine, 3,N<sup>4</sup>-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N<sup>1</sup>-dimethylcytidine, 5-(2-hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N<sup>4</sup>-methyl-N<sup>4</sup>-aminocytidine, 5-aminocytidine, 5-nitrosocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O<sup>2</sup>-methyluridine, O<sup>2</sup>-ethyluridine, 5-aminouridine, O<sup>4</sup>-methyluridine, O<sup>4</sup>-ethyluridine, O<sup>4</sup>-isobutyluridine, O<sup>4</sup>-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-

(thioalkyl)-uridine, 1,N<sup>6</sup>-ethenoadenosine, 3-methyladenosine, and N<sup>6</sup>-methyladenosine, 8-hydroxyguanosine, O<sup>6</sup>-methylguanosine, O<sup>6</sup>-ethylguanosine, O<sup>6</sup>-isopropylguanosine, 3,N<sup>2</sup>-ethenoguanosine, O<sup>6</sup>-alkylguanosine, 8-oxo-guanosine, 2,N<sup>3</sup>-ethenoguanosine, and 8-aminoguanosine.

5

32. The method of claim 30, wherein the virus is an RNA virus.

33. The method of claim 30, wherein the virus is a retrovirus or a flavivirus.

10

34. The method of claim 30, wherein the virus is selected from the group consisting of HIV-1, HIV-2, HTLV-1, HTLV-2, SIV, hepatitis A, hepatitis B, hepatitis C, BVDV, and dengue fever virus.

15

35. A method of screening for a ribonucleoside analog which is incorporated by a cellular polymerase, comprising incubating the cellular polymerase with the ribonucleoside analog in the presence of a nucleic acid template and detecting whether the ribonucleoside analog is polymerized.

20

36. The method of claim 35, wherein the cellular polymerase is present in a cell.

37. The method of claim 35, wherein the method further comprises incubating the cellular polymerase with a naturally occurring ribonucleoside.

25

38. The method of claim 35, wherein the method further comprises comparing the rate of incorporation of the ribonucleoside analog and the naturally occurring ribonucleoside into an RNA polymer.

30

39. The method of claim 35, wherein the virus is a retrovirus or a flavivirus.

40. The method of claim 35, wherein the cellular polymerase is a mammalian pol II polymerase.

41. A pharmaceutical composition comprising a therapeutically effective dose of an RNA nucleoside analog, wherein the analog is one that in a infected cell with a virus of interest is incorporated by a polymerase into an RNA copy of a genomic nucleic acid encoding the virus, said analog replacing a first natural occurring nucleotide having a first complementary nucleotide wherein said analog complements a second nucleotide which is other than the first nucleotide together with a pharmaceutically acceptable carrier.

10. 42. The pharmaceutical composition of claim 41, wherein the nucleoside analog is selected from the group consisting of N<sup>4</sup>-aminocytidine, N<sup>1</sup>-methyl-N<sup>4</sup>-aminocytidine, 3,N<sup>4</sup>-ethenocytidine, 3-methoxycytidine, 5-hydroxycytidine, N<sup>4</sup>-dimethylcytidine, 5-(2-hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N<sup>4</sup>-methyl-N<sup>4</sup>-aminocytidine, 5-aminocytidine, 5-nitrocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O<sup>2</sup>-methyluridine, O<sup>2</sup>-ethyluridine, 5-aminouridine, O<sup>4</sup>-methyluridine, O<sup>4</sup>-ethyluridine, O<sup>4</sup>-isobutyluridine, O<sup>4</sup>-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-(thioalkyl)-uridine, 1,N<sup>6</sup>-ethenoadenosine, 3-methyladenosine, and N<sup>6</sup>-methyladenosine, 8-hydroxyguanosine, O<sup>6</sup>-methylguanosine, O<sup>6</sup>-ethylguanosine, O<sup>6</sup>-isopropylguanosine, 3,N<sup>2</sup>-ethenoguanosine, O<sup>6</sup>-alkylguanosine, 8-oxo-guanosine, 2,N<sup>3</sup>-ethenoguanosine, and 8-aminoguanosine.

25. 43. The pharmaceutical composition of claim 41, wherein the pharmaceutical composition is suitable for oral administration.

44. The pharmaceutical composition of claim 41, wherein the pharmaceutical composition is suitable for parenteral administration.

30. 45. A method of increasing the mutation rate of a virus in an animal comprising administering to the animal a therapeutically effective dose of a mutagenic ribonucleoside analog composition of claim 41.

46. The method of claim 45, wherein the nucleoside analog is selected from the group consisting of N<sup>4</sup>-aminocytidine, N<sup>1</sup>-methyl-N<sup>4</sup>-aminocytidine, 3,N<sup>4</sup>-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N<sup>4</sup>-dimethylcytidine, 5-(2-5 hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N<sup>4</sup>-methyl-N<sup>4</sup>-aminocytidine, 5-aminocytidine, 5-nitrosocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O<sup>2</sup>-methyluridine, O<sup>2</sup>-ethyluridine, 5-aminouridine, O<sup>4</sup>-methyluridine, O<sup>4</sup>-ethyluridine, O<sup>4</sup>-isobutyluridine, O<sup>4</sup>-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-10 (thioalkyl)-uridine, 1,N<sup>6</sup>-ethenoadenosine, 3-methyladenosine, and N<sup>6</sup>-methyladenosine, 8-hydroxyguanosine, O<sup>6</sup>-methylguanosine, O<sup>6</sup>-ethylguanosine, O<sup>6</sup>-isopropylguanosine, 3,N<sup>2</sup>-ethenoguanosine, O<sup>6</sup>-alkylguanosine, 8-oxo-guanosine, 2,N<sup>3</sup>-ethenoguanosine, and 8-aminoguanosine.

15 47. The method of claim 45, wherein the RNA nucleoside analog is incorporated by a polymerase present in virally infected cells of the animal into an RNA copy of a genomic nucleic acid of the virus with an efficiency at least about 0.1% that of a naturally occurring complementary nucleic acid.

20 48. The method of claim 45, wherein the animal is a human patient infected with a virus selected from the group consisting of HIV-1, HIV-2, HTLV-1, HTLV-2, hepatitis A, hepatitis B, hepatitis C, and dengue fever virus.

49. The method of claim 45, wherein the animal is a human patient having 25 a disease selected from the group consisting of AIDS, hepatitis B, hepatitis C, T-cell leukemia.

50. The method of claim 45, the animal having a disease selected from the group consisting of feline leukemia virus, feline immunodeficiency virus, BVDV, or 30 vesicular stomatitis virus.

51. A method of identifying a mutagenic ribonucleoside analog,

comprising:

- providing a plurality of ribonucleoside analogs;
- incorporating a portion of the ribonucleoside analogs into a ribonucleoside polymer using a RNA polymerase;
- 5 isolating the ribonucleoside polymer; and,
- determining the chemical composition of a ribonucleoside analogs which is incorporated into the ribonucleoside polymer.

52. The method of claim 51, wherein the method further comprises  
10 hydrolyzing the ribonucleoside polymer.

53. The method of claim 51, wherein the polymer is isolated using  
electrophoresis.

54. The method of claim 51, wherein the method further comprises  
hydrolyzing the ribonucleoside polymer to yield ribonucleoside analog monomers and  
determining the structure of the ribonucleoside analog monomers using a technique selected  
from the group consisting of mass spectroscopy and NMR.

55. A method of making a mutagenic ribonucleoside analog comprising:  
chemically modifying a nucleotide analog selected from the group consisting of  
uridine, cytidine, adenosine, guanosine, N<sup>4</sup>-aminocytidine, N<sup>1</sup>-methyl-N<sup>4</sup>-aminocytidine,  
3,N<sup>4</sup>-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N<sup>4</sup>-dimethylcytidine, 5-(2-  
hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N<sup>4</sup>-methyl-N<sup>4</sup>-aminocytidine, 5-  
aminocytidine, 5-nitrosocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and  
cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O<sup>2</sup>-  
methyluridine, O<sup>2</sup>-ethyluridine, 5-aminouridine, O<sup>4</sup>-methyluridine, O<sup>4</sup>-ethyluridine, O<sup>4</sup>-  
isobutyluridine, O<sup>4</sup>-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-  
(thioalkyl)-uridine, 1,N<sup>6</sup>-ethenoadenosine, 3-methyladenosine, and N<sup>6</sup>-methyladenosine, 8-  
hydroxyguanosine, O<sup>6</sup>-methylguanosine, O<sup>6</sup>-ethylguanosine, O<sup>6</sup>-isopropylguanosine, 3,N<sup>2</sup>-  
ethenoguanosine, O<sup>6</sup>-alkylguanosine, 8-oxo-guanosine, 2,N<sup>3</sup>-ethenoguanosine, and 8-  
aminoguanosine to yield a chemically modified ribonucleoside analog;

determining whether the chemically modified analog is incorporated by an RNA polymerase into a polyribonucleotide molecule; and,

measuring the mutagenic potential of the chemically modified analog.

5       56.     The method of claim 55, wherein the step of chemically modifying the oligonucleotide comprises exposing the selected nucleotide analog to oxygen free radicals.

10      57.     A library of nucleoside analogs made by the method of claim 55, wherein each nucleoside analog comprises a random chemical substituent linked to a group selected from the group consisting of uridine, cytidine, guanosine, adenosine, N<sup>4</sup>-aminocytidine, N<sup>1</sup>-methyl-N<sup>4</sup>-aminocytidine, 3,N<sup>4</sup>-ethenocytidine, 3-methylcytidine, 5-hydroxycytidine, N<sup>4</sup>-dimethylcytidine, 5-(2-hydroxyethyl)cytidine, 5-chlorocytidine, 5-bromocytidine, N<sup>4</sup>-methyl-N<sup>4</sup>-aminocytidine, 5-aminocytidine, 5-nitrosocytidine, 5-(hydroxyalkyl)-cytidine, 5-(thioalkyl)-cytidine and cytidine glycol, 5-hydroxyuridine, 3-hydroxyethyluridine, 3-methyluridine, O<sup>2</sup>-methyluridine, O<sup>2</sup>-ethyluridine, 5-aminouridine, O<sup>4</sup>-methyluridine, O<sup>4</sup>-ethyluridine, O<sup>4</sup>-isobutyluridine, O<sup>4</sup>-alkyluridine, 5-nitrosouridine, 5-(hydroxyalkyl)-uridine, and 5-(thioalkyl)-uridine, 1,N<sup>6</sup>-ethenoadenosine, 3-methyladenosine, and N<sup>6</sup>-methyladenosine, 8-hydroxyguanosine, O<sup>6</sup>-methylguanosine, O<sup>6</sup>-ethylguanosine, O<sup>6</sup>-isopropylguanosine, 3,N<sup>2</sup>-ethenoguanosine, O<sup>6</sup>-alkylguanosine, 8-oxo-guanosine, 2,N<sup>3</sup>-ethenoguanosine, and 8-aminoguanosine.

15      58.     The library of claim 57, wherein a plurality of the nucleoside analogs are polymerized.

20      59.     The library of claim 57, wherein the library further comprises an RNA polymerase.

25      60.     The library of claim 57, wherein the library comprises between about 5 and 1,000,000 different analogs.

30      61.     The library of claim 57, wherein the cellular RNA polymerase is human RNA polymerase II.

62. A kit comprising a container and one or more of the following components: a control mutagenic RNA analog, a test mutagenic RNA analog, an RNA polymerase, reagents for detecting incorporation of the RNA analog by the RNA polymerase, and instructions in the use of the kit components for detecting the mutagenic potential of the test mutagenic analog as compared to the control mutagenic RNA analog.

63. A method of increasing the mutation rate of a virus in an animal comprising administering to the animal (a) a therapeutically effective dose of a ribonucleoside analog to a virally infected cell, wherein the analog is incorporated by a polymerase into an RNA copy of a genomic nucleic acid encoding the virus, said analog replacing a first natural occurring nucleotide having a first complementary nucleotide wherein said analog complements a second nucleotide which is other than the first nucleotide, in combination with (b) a drug that reduces the concentration of the first natural occurring nucleotide.

64. A method of increasing the mutation rate of a virus, comprising administering a free base selected from the group comprising adenine, cytosine, guanine, uracil and thymine to a virally infected cell, wherein the base is incorporated by a polymerase into an RNA or DNA copy of a genomic nucleic acid encoding the virus, said base replacing a first natural occurring nucleotide having a first complementary nucleotide wherein said base complements a second nucleotide which is other than the first nucleotide, thereby inducing the virus to mutate.

65. The ribonucleoside analog identified by the method of claim 51.

66. The method of claim 1, wherein the RNA nucleoside analog is an enantio-specific nucleoside analog.

67. The pharmaceutical composition of claim 41, wherein the RNA nucleoside analog is an enantio-specific nucleoside analog.